Image. AF | GP 1653

BEFORE THE BOARD OF APPEALS AND INTERFERENCES
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| In re application of: Glazer et al. | Group Art Unit: 1653 |
| Serial No. 09/919,486 | Examiner: Robinson, Hope |
| Filed: July 31, 2001 | Attorney Docket No. B01-114-1 |
| For: *Engineering of Living Cells for the Expression of Holo-Phycobiliprotein-Based Constructs* | CERTIFICATE OF MAILING<br>I hereby certify that this corr. is being deposited with the US Postal Service as First Class Mail in an envelope addressed to the Comm. for Patents, PO Box 1450, Alexandria, VA 22313-1450 on December 5, 2003.<br>Signed ____<br>Richard Osman |

## BRIEF ON APPEAL

The Honorable Board of Appeals and Interferences
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Honorable Board:

This is an appeal from the November 26, 2003 final rejection of claims 1-14 and 16-18.

## REAL PARTY IN INTEREST

The real party in interest is The Regents of the University of California.

## RELATED APPEALS AND INTERFERENCES

Appellants are unaware of any related appeals or interferences.

## STATUS OF THE CLAIMS

Claims 1-22 are pending and subject to this appeal.

## STATUS OF THE AMENDMENTS

All Amendments are believed to be properly before the Board.

## SUMMARY OF THE INVENTION

The field of the invention is recombinant cells engineered to express heterologous holo-phycobiliproteins. Phycobiliproteins are a family of light-harvesting proteins found in cyanobacteria, red algae, and the cryptomonads. These proteins absorb strongly in the visible region of the spectrum because they carry various covalently attached linear tetrapyrrole prosthetic groups (bilins). Phycobiliproteins are tightly associated $\alpha\beta$ heterodimers, in which each subunit carries bilin(s) thioether-linked to particular cysteinyl residues. Prior to the present invention, holophycobiliprotein expression was limited to the cyanobacteria, red algae and cryptomonads that naturally express these proteins, severely limiting their commercial application. Various steps involved in bilin biosynthesis and bilin addition to apophycobiliprotein subunits had been inferred, but never had anyone disclosed or suggested engineering cells to express recombinant enzymes sufficient to reconstitute a holophycobiliprotein biosynthetic pathway as claimed. Specification, p.1, lines 12-24.

Our claims require a recombinant cell comprising a reconstituted holophycobiliprotein biosynthetic pathway. In particular, (a) the required cell must express a holo-phycobiliprotein fusion protein comprising a heterologous-to-the-cell, fluorescent, first holo-phycobiliprotein domain fused to a heterologous protein domain, and (b) the required cell must make and comprise components: a bilin, a recombinant bilin reductase, an apo-phycobiliprotein fusion protein precursor of the fusion protein comprising a corresponding apo-phycobiliprotein domain, and a recombinant phycobiliprotein domain-bilin lyase, which components react inside the cell to form the holo-phycobiliprotein fusion protein. Specification, p.2, lines 10-19.

The present invention teaches how to engineer cells to express recombinant enzymes sufficient to reconstitute a holophycobiliprotein biosynthetic pathway, allowing recombinant protein expression in more convenient cellular expression systems. Inter alia, Specification, p.1, line 25 - p.2, line 7.

Shortly after our filing date, we published descriptions of our reconstitution of two distinct holophycobiliprotein biosynthetic pathways in heterologous cells (the cited Tooley, Cai and Glazer, Proc. Natl. Acad. Sci. USA. 2001 Sept 11, 98 (19), 10560–65; and Tooley and Glazer, J Bacteriol, Sept 2002, 184 (17), 4666-4671). In addition, following the filing and publication of our protocols, two other laboratories reported analogous reconstitution of

biosynthetic pathways for holophytochromes (the cited Landgraf et al., 2001, FEBS Letters 508, 459-62, and Gambetta and Lagarias Proc. Natl. Acad. Sci. USA. 2001 Sept 11, 98 (19), 10566–71). Phytochromes are biliprotein photoreceptors found in plants and chlorophyll-containing prokaryotes. Like the light-harvesting phycobiliproteins, phytochromes possess thioether-linked linear tetrapyrrole (bilin) prosthetic groups that enable them to absorb visible light.

## ISSUES

I. WHETHER THE EXAMINER'S REJECTION OF CLAIMS 1-14 AND 16-18 UNDER 35USC112, SECOND PARAGRAPH IS CORRECT.

II. WHETHER THE EXAMINER'S REJECTION OF CLAIMS 5-9 UNDER 35USC112, SECOND PARAGRAPH IS CORRECT.

III. WHETHER THE EXAMINER'S REJECTION OF CLAIMS 1-14 AND 16-18 UNDER 35USC103(a) IS CORRECT.

## GROUPING OF THE CLAIMS

For Issue I, claims 1, 3-14 and 16-18 stand as a group; and claims 2-4 stand as a separate group.

For Issue II, claims 5-9 stand together.

For Issue III, claims 1-14 and 16-18 stand together.

## ARGUMENT

I. CLAIMS 1-14 AND 16-18 ARE PATENTABLE UNDER 35USC112, SECOND PARAGRAPH

The claims are definite as written.

In claim 1, the recited heterologous fusion protein is the displayed domain as described inter alia, on p.7, line 19 - p.10, line 8.

In claim 1, the cell "makes and comprises" all the recited components: a bilin, a

recombinant bilin reductase, an apo-phycobiliprotein fusion protein precursor of the fusion protein comprising a corresponding apo-phycobiliprotein domain, and a recombinant phycobiliprotein domain-bilin lyase.

Claim 1 is not incomplete, as the required bilin must be present in the cell. Dependent claims 2-4 require that the required bilin be formed by a reaction in the cell between a resident heme and a resident heme oxygenase. Hence, even under the Action's misconstruction of claim 1, these claims should not have been included in this aspect of the rejection.

We note the Examiner's proposed revised claim 1 appears to combine the limitations of pending claims 1 and 6. However, the Specification thoroughly teaches heterologous protein domains (displayed domains) that are neither fluorescent nor comprise a second holophycobiliprotein domain. See, inter alia, p.7, line 19 - p.10, line 8.

## II. CLAIMS 5-9 ARE PATENTABLE UNDER 35USC112, SECOND PARAGRAPH

The claims are definite as written.

Claims 5-9 require that the heterologous protein domain is fluorescent and spectroscopically distinguishable from the first holo-phycobiliprotein domain. The claims do not require any particular difference in fluorescence, nor are they dependent on operator or equipment variation. So long as the heterologous protein domain is spectroscopically distinguishable from the first holo-phycobiliprotein domain, the limitation is met.

## III. CLAIMS 1-14 AND 16-18 ARE PATENTABLE UNDER 35USC103(a)

The Applicants are intimately familiar with the cited Fairchild (1992, PNAS 89:7017-21), Allnutt (US Pat/PUB No. 20010055783, June 16, 2001) and Frankenberg (2001, The Plant Cell 13:965-78) references. Fairchild describes in vitro phycocyanobilin lyase activity. The investigators show that a crude and fractionated cyanobacterial extract can catalyze bilin addition to the alpha subunit of apophycocyanin (apo-$\alpha$PC), and that recombinant subunits of the heterodimeric phycocyanobilin lyase, the recombinant CpCE and CpcF proteins, enhanced addition (e.g. Fig. 2). They also show that cyanobacteria-expressed holophycocyanin could serve as a bilin donor in these reactions (e.g. Fig. 3). Nowhere does this reference teach or suggest that a holophycobiliprotein could be expressed in any cell other than a cyanobacterium

that naturally expresses such proteins. Fairchild does not teach or suggest modifying E. coli to make a holophycobiliprotein. Fairchild does express apo-phycobiliproteins in E. coli, but when they need a cell to make the holo-form, they exclusively teach expression in cyanobacteria (e.g. p.7018, col.2, lines 36-62).

As explained in our Specification, Fairchild is one of several early reports which inferred steps involved in bilin biosynthesis and bilin addition to apophycobiliprotein subunits in cyanobacteria (Specification, p.1, lines 21-22). However, none of these reports teach or suggest the feasibility of engineering cells to express recombinant enzymes sufficient to reconstitute a holophycobiliprotein biosynthetic pathway.

Allnutt describes using developed methods for manipulating cyanobacteria to express phycobiliprotein fusion proteins. Because there was no other way of making holophycobiliproteins at the time, Allnutt describes expressing these proteins exclusively in cyanobacteria (e.g. Allnutt Examples 1-12). Nowhere does this reference teach or suggest that a holophycobiliprotein could be expressed in any cell other than a cyanobacterium that naturally expresses such proteins, but like Farchild, when Allnutt needs a cell to make a holophycobiliprotein, they exclusively teach expression in cyanobacteria.

Frankenberg (2001, The Plant Cell 13:965-78) identified genes encoding bilin reductases that catalyze the ferredoxin-dependent reduction of biliverdin IXα to phytobilins. As explained in our Specification, Frankenberg is one of several early reports which inferred steps involved in bilin biosynthesis and bilin addition to apophycobiliprotein subunits in cyanobacteria (Specification, p.1, lines 21-22). Of course, these reports provide necessary background information on enzymes that might ultimately prove necessary to reconstitute a holophycobiliprotein biosynthetic pathway. However, simply disclosing that this or that enzyme may be involved in the natural holophycobiliprotein biosynthetic pathway does not teach or suggest the feasibility of engineering cells to express recombinant enzymes sufficient to reconstitute a holophycobiliprotein biosynthetic pathway.

As noted above, our claims require a recombinant cell comprising a reconstituted holophycobiliprotein biosynthetic pathway. The required cell must (a) express a holo-phycobiliprotein fusion protein comprising a heterologous-to-the-cell, fluorescent, first holo-phycobiliprotein domain fused to a heterologous protein domain, and (b) make and comprise

components: a bilin, a recombinant bilin reductase, an apo-phycobiliprotein fusion protein precursor of the fusion protein comprising a corresponding apo-phycobiliprotein domain, and a recombinant phycobiliprotein domain-bilin lyase, which components react inside the cell to form the holo-phycobiliprotein fusion protein. Prior to our disclosure, no one had disclosed or suggested engineering cells to express recombinant enzymes sufficient to reconstitute a holophycobiliprotein biosynthetic pathway as claimed.

Appellants respectfully request reversal of the pending Final Action by the Board of Appeals.

We petition for and authorize charging our Deposit Account No.19-0750 all necessary extensions of time. The Commissioner is authorized to charge any fees or credit any overcharges relating to this communication to our Dep. Acct. No.19-0750 (order B01-114).

Respectfully submitted,
SCIENCE & TECHNOLOGY LAW GROUP

Richard Aron Osman, J.D., Ph.D., #36,627
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Mission Statement, USPTO External Customer Services Guide

CLAIMS ON APPEAL

1. A recombinant cell which expresses a holo-phycobiliprotein fusion protein comprising a heterologous-to-the-cell, fluorescent, first holo-phycobiliprotein domain fused to a heterologous protein domain, wherein the cell makes and comprises components: a bilin, a recombinant bilin reductase, an apo-phycobiliprotein fusion protein precursor of the fusion protein comprising a corresponding apo-phycobiliprotein domain, and a recombinant phycobiliprotein domain-bilin lyase, which components react inside the cell to form the holo-phycobiliprotein fusion protein.

2. The cell of claim 1, wherein the cell further comprises a heme and a heme oxygenase which react to form the bilin.

3. The cell of claim 1, wherein the cell further comprises a heme and a recombinant heme oxygenase which react to form the bilin.

4. The cell of claim 1, wherein the cell further comprises a heme and a recombinant heme oxygenase which react to form the bilin, and the recombinant heme oxygenase is HO1.

5. The cell of claim 1, wherein the heterologous protein domain is fluorescent and spectroscopically distinguishable from the first holo-phycobiliprotein domain.

6. The cell of claim 1, wherein the heterologous protein domain is fluorescent and spectroscopically distinguishable from the first holo-phycobiliprotein domain, and the heterologous protein domain comprises a heterologous-to-the-cell, fluorescent, second holo-phycobiliprotein domain.

7. The cell of claim 1, wherein the heterologous protein domain is fluorescent and spectroscopically distinguishable from the first holo-phycobiliprotein domain, and the heterologous protein domain comprises a phytochrome domain.

8. The cell of claim 1, wherein the heterologous protein domain is fluorescent and

spectroscopically distinguishable from the first holo-phycobiliprotein domain, and the heterologous protein domain comprises a green fluorescent protein (GFP) domain.

9. The cell of claim 1, wherein the heterologous protein domain is fluorescent and spectroscopically distinguishable from the first holo-phycobiliprotein domain, and the fusion protein provides fluorescence resonance energy transfer between the first holo-phycobiliprotein domain and the heterologous protein domain.

10. The cell of claim 1, wherein the cell is a mammalian cell.

11. The cell of claim 1, wherein the cell is a yeast cell.

12. The cell of claim 1, wherein the cell is a bacterial cell.

13. The cell of claim 1, wherein the cell is an *Escherichia coli* cell.

14. The cell of claim 1, wherein the cell is *in vitro*.

16. The cell of claim 1, wherein the bilin is phycocyanobilin (PCB), the reductase is 3Z-phycocyanobilin:ferredoxin oxidoreductase (PcyA), the apo-phycobiliprotein domain is phycocyanin $\alpha$ subunit domain, and the lyase is heterodimeric phycocyanin $\alpha$ subunit phycocyanobilin lyase (CpcE and CpcF).

17. The cell of claim 1, wherein the bilin is phycocyanobilin (PCB), the reductase is 3Z-phycocyanobilin:ferredoxin oxidoreductase (PcyA), the apo-phycobiliprotein domain is phycoerythrocyanin apo-$\alpha$ subunit domain, and the lyase is heterodimeric phycoerythrocyanin $\alpha$ subunit phycoerythrocyanobilin lyase (PecE and PecF), which further catalyzes the isomerization of the bilin to phycobiliviolin.

18. The cell of claim 1, wherein the bilin is phycoerythrobilin (PEB), the reductase is 3Z-

phycoerythrobilin:ferredoxin oxidoreductase (PebA and PebB), the apo-phycobiliprotein domain is phycoerythrin apo-$\alpha$ subunit domain, and the lyase is heterodimeric C-phycoerythrin $\alpha$ subunit phycoerythrobilin lyase (CpeY and CpeZ).

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As noted above, our claims require a recombinant cell comprising a reconstituted holophycobiliprotein biosynthetic pathway. The required cell must (a) express a holo-phycobiliprotein fusion protein comprising a heterologous-to-the-cell, fluorescent, first holo-phycobiliprotein domain fused to a heterologous protein domain, and (b) make and comprise

components: a bilin, a recombinant bilin reductase, an apo-phycobiliprotein fusion protein precursor of the fusion protein comprising a corresponding apo-phycobiliprotein domain, and a recombinant phycobiliprotein domain-bilin lyase, which components react inside the cell to form the holo-phycobiliprotein fusion protein. Prior to our disclosure, no one had disclosed or suggested engineering cells to express recombinant enzymes sufficient to reconstitute a holophycobiliprotein biosynthetic pathway as claimed.

Appellants respectfully request reversal of the pending Final Action by the Board of Appeals.

We petition for and authorize charging our Deposit Account No.19-0750 all necessary extensions of time. The Commissioner is authorized to charge any fees or credit any overcharges relating to this communication to our Dep. Acct. No.19-0750 (order B01-114).

Respectfully submitted,
SCIENCE & TECHNOLOGY LAW GROUP

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Mission Statement, USPTO External Customer Services Guide

## CLAIMS ON APPEAL

1. A recombinant cell which expresses a holo-phycobiliprotein fusion protein comprising a heterologous-to-the-cell, fluorescent, first holo-phycobiliprotein domain fused to a heterologous protein domain, wherein the cell makes and comprises components: a bilin, a recombinant bilin reductase, an apo-phycobiliprotein fusion protein precursor of the fusion protein comprising a corresponding apo-phycobiliprotein domain, and a recombinant phycobiliprotein domain-bilin lyase, which components react inside the cell to form the holo-phycobiliprotein fusion protein.

2. The cell of claim 1, wherein the cell further comprises a heme and a heme oxygenase which react to form the bilin.

3. The cell of claim 1, wherein the cell further comprises a heme and a recombinant heme oxygenase which react to form the bilin.

4. The cell of claim 1, wherein the cell further comprises a heme and a recombinant heme oxygenase which react to form the bilin, and the recombinant heme oxygenase is HO1.

5. The cell of claim 1, wherein the heterologous protein domain is fluorescent and spectroscopically distinguishable from the first holo-phycobiliprotein domain.

6. The cell of claim 1, wherein the heterologous protein domain is fluorescent and spectroscopically distinguishable from the first holo-phycobiliprotein domain, and the heterologous protein domain comprises a heterologous-to-the-cell, fluorescent, second holo-phycobiliprotein domain.

7. The cell of claim 1, wherein the heterologous protein domain is fluorescent and spectroscopically distinguishable from the first holo-phycobiliprotein domain, and the heterologous protein domain comprises a phytochrome domain.

8. The cell of claim 1, wherein the heterologous protein domain is fluorescent and

spectroscopically distinguishable from the first holo-phycobiliprotein domain, and the heterologous protein domain comprises a green fluorescent protein (GFP) domain.

9. The cell of claim 1, wherein the heterologous protein domain is fluorescent and spectroscopically distinguishable from the first holo-phycobiliprotein domain, and the fusion protein provides fluorescence resonance energy transfer between the first holo-phycobiliprotein domain and the heterologous protein domain.

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16. The cell of claim 1, wherein the bilin is phycocyanobilin (PCB), the reductase is 3Z-phycocyanobilin:ferredoxin oxidoreductase (PcyA), the apo-phycobiliprotein domain is phycocyanin α subunit domain, and the lyase is heterodimeric phycocyanin α subunit phycocyanobilin lyase (CpcE and CpcF).

17. The cell of claim 1, wherein the bilin is phycocyanobilin (PCB), the reductase is 3Z-phycocyanobilin:ferredoxin oxidoreductase (PcyA), the apo-phycobiliprotein domain is phycoerythrocyanin apo-α subunit domain, and the lyase is heterodimeric phycoerythrocyanin α subunit phycoerythrocyanobilin lyase (PecE and PecF), which further catalyzes the isomerization of the bilin to phycobiliviolin.

18. The cell of claim 1, wherein the bilin is phycoerythrobilin (PEB), the reductase is 3Z-

phycoerythrobilin:ferredoxin oxidoreductase (PebA and PebB), the apo-phycobiliprotein domain is phycoerythrin apo-α subunit domain, and the lyase is heterodimeric C-phycoerythrin α subunit phycoerythrobilin lyase (CpeY and CpeZ).